PHYSICOCHEMICAL, MICROBIOLOGICAL AND COLOUR ATTRIBUTES OF HORSE SALAMI ESTABLISHED DURING THE RIPENING PERIOD

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ABSTRACT

Changes in physicochemical, colour, textural, microbiological and sensory attributes occurring during the processing of Horse Salami and established on manufacturing days 0, 7, 14, 21, 28, 42, 60, 90 were studied. Significant changes (P<0.05) in physicochemical parameters attributable to moisture loss, as well as changes in colour and textural properties were observed during the fermentation and ripening stage. Proteolysis and lipolysis, coming as a result of endogenous enzymatic activity and high lactic acid bacteria and staphylococci counts, contributed to specific organoleptic properties of the final product. Sensorial profiling showed a significant (P<0.05) acid taste, lactic acid odour and flavour intensity, and low fat/lean ratio and smokiness and saltiness values. Final Horse Salami products were microbiologically safe, the dominant microbial population thereby being Lactobacillus plantarum, Lactococcus lactis ssp. lactis, Enterococcus faecium and Staphylococcus xylosus.

Keywords: CIE L*, a*, b*, horse Salami, microbiological aspect, physicochemical properties, Sensory attributes, texture profile analysis
INTRODUCTION

Horse Salami, an indigenous Croatian meat product, is a dry fermented sausage made of horse meat supplemented with pork fatback, salt and spices. In Croatia, the tradition of Horse Salami production is kept by the Italian minority populating the eastern part of the Country (in specific, the western Slavonian region). In the past, this product had been known as “the dish of the poor”; nowadays, it represents a highly appreciated autochthonous Croatian meat product having a great potential to become a PGI (Protected Geographical Indications) & PDO (Protected Designation of Origin). Although horse meat has a high nutrition, as well as a high mineral value (due to its vitamin B and iron content, respectively) (BADIANI et al., 1997; FRANCO et al., 2011), human consumption is negligible in comparison with other conventional types of meat like pork, beef or chicken (LOMBARDI et al., 2005). Horse meat used for the production of Horse Salami is obtained from horses slaughtered at the end of their (5-year or longer) lifecycle. The meat has no appreciable organoleptic qualities. Its original colour is deep red larded with yellow fat, while the meat is tough to chew due to the connective tissue maturation (LJTWINiczUK et al., 2008; TATEO et al., 2008).

Horse Salami has specific sensorial properties (smell and taste) attributable mainly to drying and smoking, but also to ripening, as well as to enzymatic, lactic acid bacteria and mould activity. The recipe is 130 years old and the sole difference in final products coming from various producers boils down to the difference in mass fraction of fatback used in the Salami preparation (ranging from 12 to 15%). The production of the traditional Horse Salami mainly takes place on small farms; we are therefore talking a small-scale production seasonal in its nature, fluctuating on a year-by-year basis dependent on weather conditions. In light of the foregoing, standardization of the Horse Salami production becomes imperative. Dry sausages produced in various European countries, mainly Spain and Italy, have been extensively studied for their physicochemical composition, colour and textural properties (CASIRAGHI et al., 1996; GIMENO et al., 2000; BRUNA et al., 2001; SPAZIANI et al., 2009). However, scientific information on this Croatian indigenous dry sausage, which would efficiently contribute to its characterization and production standardisation, is virtually non-existent.

Therefore, the aim of this study was to investigate, for the first time ever, physicochemical composition, microbiological and sensorial attributes of the dry-fermented sausage known as Horse Salami and the changes occurring during 90 days of its manufacturing. Investigations also included instrumental measurements of colour and texture of the studied Salami on certain processing days, as well as the isolation and identification of autochthonous microbial population and gathering of other data needed for microbiological safety evaluation of the final product.

MATERIAL AND METHODS

The manufacturing process

Samples of traditional Horse Salami (24 units) were manufactured in a small-scale facility in the western Slavonian region (the Eastern Croatia). All samples were prepared using traditional procedures that made no use of additives such as starter cultures supplemented with nitrates, nitrates or ascorbic acid (namely, the production of traditional Croatian meat products does not involve the use of additives). Such a traditional production takes about 3 months (90 days). Horse Salami is made of meat of older (5+ years), worn-out horses, mainly of the Hrvatski Posavac breed. After slaughtering, fat and connective tissue are carefully removed from the horse meat. This is especially important when it comes to fat, because horse fat has a particularly unpleasant smell and taste. The meat is then grinded using a grinding plate having holes measuring 6 mm in their diameter and left to rest overnight (12 hours at the minimum) in a special container equipped with a decantation hole. Grinded horse meat is then mixed with pig fatback represented in the amount of 12%. Before its mixing with the horse meat, the fatback is grinded using a grinding plate having holes measuring 10 mm in their diameter. The mixture of meat and fat is then mixed with salt added in the amount of 2.2%, red paprika powder added in the amount of 0.2%, hot red paprika powder added in the amount of 0.3%, garlic added in the amount of 0.2%, and black pepper added in the amount of 0.3%. In the subsequent course, the mixture gets to be stuffed into a horse small intestine (roughly 50 cm long and 50 mm wide in diameter) or into collagen casings (of the same dimensions). Thereafter, the Horse Salami is smoked on a dry hard wood (hornbeam, beech and its sawdust) every few days (for 2-3 hours) for the total of four weeks. At this stage, the temperature and relative humidity should be kept at 18 to 20°C and 70 to 90%, respectively. After smoking, the Horse Salami is left to ripen. This stage is the longest and should take about two months, throughout which period the Salami should be kept in a dark room at the temperature ranging from 14° to 17°C, with the relative humidity ranging from 70 to 80%. After that, Horse Salami is ready for consumption. Within this study frame, samples of Horse Salami were taken on the processing days 0, 7, 14, 21, 28, 42, 60 and 90. In total, 24 samples were produced; at each processing stage, three samples were taken for the analyses.
ANALYTICAL METHODS

Physicochemical parameters

Before the analysis, the Sausage samples were homogenised using a knife mill Gridomix GM 200 (Retsh, Germany) and prepared according to ISO 3100-1:1975.

Water content was determined gravimetrically (ISO 1442:1997) at 103°C (Epsa 2000 Bari, Croatia), while the ash content was established according to ISO 936:1998, by virtue of burning the samples at 550°C (LV9/11/P320 Nobtherm, Germany). Total protein content was determined using the Kjeldahl method (ISO 937:1978) that made use of an Unit 8 Basic digestion block (Foss, Sweden) and a Kjeltec 8400 automated distillation & titration device (Foss, Sweden). The total fat content was determined using the Soxhlet method (ISO 1443:1973), which involves digestion of a sample in a acidic environment followed by fat extraction with petroleum ether using a Soxtherm 2000 Automatic device (Gerhardt, Germany). The determination of collagen content was performed through the analysis of hydroxyproline according to ISO 3496:1994 that made use of a spectrophotometer (Hach DR/4000U, Germany). Sodium chloride content was determined using the internal titration method (TRAJKOVIĆ et al., 1983). In this analysis, 2 g of each sample were homogenized with sand and 3 mL of water. The content was transferred into a 100 mL-volumetric flask, stirred and placed for 15 min into a water bath at 100°C. After cooling, the flask was filled with water up to the mark and filtered. An aliquot (25 mL) of the filtrate was transferred into an Erlenmeyer flask containing a few drops of K₂CrO₄ indicator (62 g/100 mL of water) and titrated with 0.1 M AgNO₃, until a persistent reddish colour was obtained. Sodium chloride content was calculated based on the expenditure of titration reagent and its concentration.

pH values were determined in a homogenate diluted with distilled water (1:10, p/v) using pH/Ion 510 – Bench pH/Ion/mV Meter (Eutech Instruments Pte Ltd/ Oakton Instruments, USA) according to the pH/Ion 510 Instruction Manual. Water activity (aₓ) was determined at the room temperature (20±2°C) using a Rotronic HygroLab 3 (Rotronic AG, Bassersdorf, Switzerland). All chemicals used for analyses of physicochemical parameters were of an analytical grade. For each sample, three independent measurements were made.

Instrumental determination of colour

Instrumental colour measurements (those of L*, a* and b* values) were performed using a Hunter-Lab Mini ScanXE (A60-1010-615 Model Colorimeter, Hunter-Lab, Reston, VA, USA). The instrument was standardized on each occasion using a white ceramic plate (L₀ = 93.01, a₀ = -1.11, and b₀ = 1.30). The CIELAB space values (L*, a* and b*) (CIE, 1976) correspond to lightness, greenness (-a*), redness (a*), blueness (-b*) or yellowness (b*). The colour measurements performed on the Horse Salami took place at the room temperature (20±2°C). Each sample was cut in slices and colour-measured at ten different spots.

Texture Profile Analysis

Texture Profile Analysis (TPA) was performed using a TA.XT2i SMS Stable Micro Systems Texture Analyzer (Stable Microsystems Ltd, Surrey, England) equipped with a P/75 aluminium cylindrical probe. This involved cutting the samples into 1.5 cm-thick slices and their double compression so as to downsize them to 40% of their original thickness. Force-time curves were recorded at the across-head speed of 5 mms⁻¹ and at the same recording speed. The following parameters were quantified (BOURNE, 1978): hardness (kg), i.e. the maximum force required to compress the sample; springiness (ratio), i.e. the ability of the sample to recover its original form after the cessation of the deforming force; cohesiveness (ratio), i.e. the extent to which the sample could be deformed prior to rupture; chewiness (kg), i.e. labour required to masticate the sample before swallowing, which represents the product of hardness multiplied by cohesiveness and springiness; and finally resilience (ratio), so as to determine how well the product "fights to regain its original position". These parameters were obtained using the Texture Expert for Windows (Version 1.0) Stable Micro Systems. With each sample, eight determinations of texture parameters were made.

Microbiological analysis

After aseptically removing and discarding the casing, 10 g of the product were recovered in an aseptic manner, homogenized in 90 ml of the sterile 0.5%-saline solution and serially diluted before their planting on a non-selective (peptide yeast extract glucose agar, Biolife, Milano, Italy), PCA-agar (standard plate count agar) (Biolife, Milano, Italy) and the following selective media: MRS-agar (Biolife, Milano, Italy) intended for lactic acid bacteria growth and Baird-Parker agar (Merck, Darmstadt, Germany) intended for staphylococci growth. The plates were incubated under conditions specified in Table 1.

Isolation and identification of microbial population in the final product

Classical microbiological and biochemical (API) methods (Table 1) were used for the isolation and identification of the natural microbial population in the traditionally produced Horse
salami (i.e. in the final product obtained after 90 production days). Ten grams of the sample were homogenized in 90 mL of sterile 0.5% saline solution and serially diluted before planting on a non-selective medium (peptone yeast extract glucose agar, Biolife, Milano, Italy) and selective media under conditions specified in Table 1. Colonies randomly taken from selected plates were identified on the basis of their morphology, Gram-staining, cell morphology and catalase reaction. The identity of bacteria species was further confirmed using the API identification kits (BioMérieux, France).

### Table 1 - Classical microbiological and biochemical (API) methods of isolation and identification of microbial population applied in the Horse Salami analyses.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Nutrient media</th>
<th>Incubation conditions</th>
<th>API test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella sp.</td>
<td>RP-broth, XLD</td>
<td>37°C 24-48 h</td>
<td>API 20 E V4.1</td>
</tr>
<tr>
<td></td>
<td>(Biolife, Italy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>VRBG</td>
<td>37°C 24 h</td>
<td>API 20 E V4.1</td>
</tr>
<tr>
<td></td>
<td>(Biolife, Italy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>BP</td>
<td>37°C 48 h</td>
<td>API Staph V4.1</td>
</tr>
<tr>
<td></td>
<td>(Biolife, Italy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coagulase negative staphylococci (CNS)</td>
<td>BP</td>
<td>37°C 48 h</td>
<td>API Staph V4.1</td>
</tr>
<tr>
<td></td>
<td>(Biolife, Italy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulphite reducing clostridia</td>
<td>Sulphite agar</td>
<td>37°C 72 h</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Biolife, Italy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Fraser broth</td>
<td>37°C 24 h</td>
<td>API Listeria V1.2</td>
</tr>
<tr>
<td></td>
<td>Palcam agar</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Biolife, Italy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>MRS agar</td>
<td>30°C 48-72 h</td>
<td>API 50 CHL V5.1</td>
</tr>
<tr>
<td></td>
<td>(Biolife, Italy)</td>
<td></td>
<td>API 20 STREP V7.0</td>
</tr>
<tr>
<td>Yeasts</td>
<td>Sabouraud agar</td>
<td>25°C 48-72 h</td>
<td>API 20 C AUX V4.0 Yeasts</td>
</tr>
<tr>
<td></td>
<td>(Biolife, Italy)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sensorial analysis

The final Horse Salami product (obtained after 90 days) was subjected to a quantitative descriptive analysis performed by a panel of seven (3 male and 4 female) trained experts according to ISO 6658:2005 standard. The panellists had completed a preliminary three session-training in order to familiarize themselves with the samples under investigation. Fourteen attributes were examined and rated on a 5-point scale, “1” thereby standing for “poorly perceived or absent” and “5” standing for “intensely perceived”. During these three training sessions, the descriptors to be targeted by the analysis were agreed upon. The latter included as follows: 2 external attributes (appearance, hardness), 4 attributes descriptive of a slice (fat/lean ratio, easy peeling capability, colour intensity, sliceability), 5 attributes descriptive of perceptions during mastication (flavour intensity, juiciness, smokiness, acid taste, saltiness) and 3 attributes descriptive of the product smell (spice odour, lactic acid odour, mould odour). The Sausage samples were coded using a three-digit code and presented in form of oblique slices approximately 0.4 cm thick. Water was provided to clean the panellists’ palate between analyses.

Data analysis

Differences between the average values of the same physicochemical, colour, texture, microbiological and sensory parameters were analyzed using the analysis of variance (ANOVA) and the Fisher’s least significant difference test (LSD), with statistical significance being set at $P<0.05$. Moisture, fat, protein, collagen and NaCl content, pH, aw, colour and textural parameters were subjected to correlation analysis (Pearson’s correlation test) so as to determine their possible statistically meaningful relationships. Statistical analysis was carried out using Statistica Ver. 8.0 StatSoft Inc. Tulsa, OK, USA.
RESULTS AND DISCUSSION

Physicochemical parameters

Basic chemical composition, salt (NaCl) content, pH values and water activity (a_w) of the Horse Salami, established at various processing stages, are given in Table 2. The average initial moisture content of the Horse Salami found to be 61.91% had significantly decreased (P<0.05) as the processing went on due to smoking and dry-ripening typical of dry fermented sausages (LiZAsO et al., 1999; PEREZ-ALVAREZ et al., 1999; SALGADO et al., 2005; SALGADO et al., 2006; LORENZO et al., 2012). Higher moisture losses were observed in the first 21 processing days and on day 28, which is characteristic for this type of product (< 40%) and dry sausages in general (PLEADIN et al., 2014). Further ripening leads to additional moisture content reduction, so that the lowest value (28.51%) was determined on manufacturing day 90. In 2012, LORENZO and co-workers reported higher initial and final moisture values for the foal salchichon. This can be explained by the fact that horse meat has a lower water content as compared to foal meat (LITWINCZUK et al., 2008; LANZA et al., 2009; TATEO et al., 2008), as well as by the longer ripening period of the Horse Salami. The final moisture content was also lower than in similar dry sausages coming from Spain (GI-MENO et al., 2000; RUBIO et al., 2007; LORENZO et al., 2012), which can also be attributed to a longer ripening period of the Horse Salami.

The highest amount of proteins (30.53%) was determined on day 90. The results are consistent with the published literature data, which show that due to prolonged drying and ripening (weight loss of up to 50%) and a high share of lean meat used in stuffing preparation, moisture and protein content in ripened dry-fermented sausages tend to be similar (30-40%), indicating a high nutritional value of the final product (PLEADIN et al., 2014).

The average fat content of the Horse Salami had increased significantly (P<0.05) from day 1 to day 90 (from 13.84 to 28.54%), in proportion to the duration of the Horse Salami ripening process and dehydration, i.e. the continuous reduction of water content in the product; the same goes for the protein and collagen content (Table 2). Fat as a substantial component of fermented sausages has multiple functions; it represents a concentrated energy source (9 kcal/g) and the source of essential fatty acids and fat-soluble vitamins (MELA, 1990). Furthermore, it is contributing to the fullness of flavour, texture and softness of the product, all of the aforementioned being relevant for the quality and acceptability of the product in question (OLIVARES et al., 2010). Hydrolysis and oxidation of fatty acids that occur during the ripening process largely contribute to the taste of fermented sausages (ORDONEZ et al., 1999). The final fat content was lower, while the final protein content turned out to be higher than in Spanish and Italian dry fermented sausages (DELLAGLIO et al., 1996; RUBIO et al., 2008). The average initial ash content was 3.13% and had increased significantly (P<0.05), reaching the ultimate value of 5.72%, whereas water activity (a_w) (Table 2) had decreased significantly (P<0.05) during the smoking and dry-ripening period (from 0.96 to 0.78). Changes in mass fraction of individual basic constituents and water activity decrease seen after 90 days of Horse Salami production (Table 2) are mostly caused by the drying process, i.e. the loss of water occurring during ripening.

Changes in pH values seen during the processing of the Horse Salami are presented in Table 2. pH value had decreased during the first 21 days of processing (from 5.58 to 4.71), possibly as a result of the presence of organic acid produced by bacteria (LUCKE, 1994). This pH drop is typical of most dry fermented sausage (PEREZ-ALVAREZ et al., 1999; GIMENO et al., 2000; LIZASO et al., 1999; MUGUERZA et al., 2002; BOZKURT and BAYRAM, 2006; VAN SCHALKWYK et al., 2011). At the final processing stage, pH values increased to 4.94, possibly due to the liberation of peptides, amino acid and ammonia re-

<table>
<thead>
<tr>
<th>Processing time (days)</th>
<th>Moisture (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Collagen (%)</th>
<th>Ash (%)</th>
<th>Salt (NaCl) (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>61.91±0.06</td>
<td>13.84±0.03</td>
<td>17.05±0.04</td>
<td>0.63±0.11</td>
<td>3.13±0.02</td>
<td>2.30±0.04</td>
<td>5.58±0.03</td>
</tr>
<tr>
<td>7</td>
<td>55.67±0.01</td>
<td>15.83±0.03</td>
<td>22.48±0.01</td>
<td>1.19±0.01</td>
<td>3.73±0.06</td>
<td>2.71±0.05</td>
<td>4.99±0.03</td>
</tr>
<tr>
<td>14</td>
<td>48.92±0.09</td>
<td>17.71±0.01</td>
<td>23.73±0.01</td>
<td>1.56±0.07</td>
<td>4.56±0.04</td>
<td>3.29±0.03</td>
<td>4.74±0.10</td>
</tr>
<tr>
<td>21</td>
<td>43.22±0.06</td>
<td>18.55±0.02</td>
<td>24.36±0.08</td>
<td>2.05±0.21</td>
<td>4.67±0.04</td>
<td>3.63±0.05</td>
<td>4.72±0.08</td>
</tr>
<tr>
<td>28</td>
<td>37.57±0.01</td>
<td>18.59±0.04</td>
<td>27.62±0.04</td>
<td>2.06±0.09</td>
<td>4.94±0.01</td>
<td>3.75±0.05</td>
<td>4.76±0.06</td>
</tr>
<tr>
<td>42</td>
<td>38.16±0.04</td>
<td>20.59±0.02</td>
<td>27.95±0.05</td>
<td>2.82±0.15</td>
<td>5.36±0.05</td>
<td>3.81±0.05</td>
<td>4.81±0.10</td>
</tr>
<tr>
<td>60</td>
<td>31.61±0.06</td>
<td>25.45±0.06</td>
<td>29.34±0.23</td>
<td>2.84±0.12</td>
<td>5.45±0.06</td>
<td>4.24±0.02</td>
<td>4.93±0.07</td>
</tr>
<tr>
<td>90</td>
<td>35.16±0.04</td>
<td>28.54±0.12</td>
<td>30.53±0.23</td>
<td>3.93±0.10</td>
<td>5.72±0.01</td>
<td>4.51±0.03</td>
<td>4.59±0.03</td>
</tr>
</tbody>
</table>

Values are means ±SD obtained with three measurements. Values displayed in the same row and tagged with different letters (a-h) are significantly different (P<0.05).

Table 2 - Basic chemical composition, salt content, a_w and pH of the Horse Salami established during the manufacturing process.
sulting from a proteolytic reaction (Spaziani et al., 2009). The final pH was lower than in most dry fermented sausages (5.2 to 5.8) (Bover-Cid et al., 2001; Rubio et al., 2007; Roserio et al., 2010), which can be explained by horse meat properties in terms of higher glycogen content as compared to pork, beef and foal meat (Lawn and Ledward, 2006).

The salt content of the Horse Salami had significantly increased during processing (P<0.05) (Table 2). Literature sources have reported the average mass fraction of salt in dry sausage stuffing to range from 2.0% to 2.6%, and that in final products to range from 3.3% to 4.3% (Ockerman and Basu, 2007; Stahnke and Tjener, 2007). In this study, mass fraction of salt (NaCl) established during the Horse Salami manufacturing process ranged from 2.31% to 4.51%.

**Instrumental colour properties**

The CIELAB space (L*, a* and b*) values of the Horse Salami were significantly affected (P<0.05) by the length of smoking and ripening period (Table 3). Lower lightness L* values seen with an increased length of processing are probably related to the dark colour of the Horse Salami coming as a consequence of browning. A similar decrease in L* values during ripening was reported by Bozkurt and Bayram (2006) for Turkish sucuk, and by Lorenzo et al. (2012) for foal salchichon. Redness (a*) had significantly (P<0.05) decreased at all processing stages. Similar lower a* values were seen during the ripening of Spanish pork dry sausages and foal salchichon, as reported by Pérez-Alvarez et al. (1999) and Lorenzo et al. (2012). Lowering of a* values can possibly be explained by total or partial denaturation of nitrosomyoglobin coming as a result of lactic acid production. L* and a* values lower than those reported by Lorenzo et al. (2012) can probably be related to the nature of horse meat, which is darker and redder than foal (Taeto et al., 2008).

Yellowness (b*) had decreased from 20.32 to 9.11 and had varied significantly (P<0.05) during the production process. The decrease in b* values seen with the prolongation of the processing time was also reported by other authors (Pérez-Alvarez et al., 1999; Lorenzo et al., 2012) and explained by the decrease in concentration of oxyhemoglobin coming as a result of oxygen consumption executed by microorganisms.

**Texture Profile Analysis**

Texture Profile Analysis (TPA) parameters of the Horse Salami established during the smoking and dry ripening period are presented in Table 4. Average hardness values had significantly increased (P<0.05) from 0.32 to 20.54 kg as the processing went by. This can be related to the coagulation of muscle protein coming as a result of low pH values and sausage drying (Bozkurt and Bayram, 2006).

Springiness and cohesiveness had significantly decreased (P<0.05) during the processing (from 0.76 to 0.64 and from 0.67 to 0.43, respectively). Springiness is related to elastic properties of the meat and is an important quality indicator in dry-cured sausages. The decrease in springiness values could be attributed to the drying process and the shrinkage of the meat, which results in a harder texture.
ties, so that the decrease in this textural property of the Horse Salami is most likely to be related to the removal of water (Bozkurt and Bayram, 2006).

Increases in gumminess and chewiness values (from 0.26 to 8.83 and from 0.20 to 5.65, respectively) seen during the Horse Salami processing were statistically significant (P<0.05). Increase in chewiness values indicates that the Horse Salami becomes tougher during the ripening period (Szczesniak, 2002), possibly due to moisture loss.

Resilience values established at the beginning and at the end of the processing were 0.19 and 0.14, respectively. Significant changes in resilience during smoking and ripening failed to be observed (P>0.05) (Table 4).

**Microbial counts**

Microbial flora changes seen during manufacturing are shown in Fig. 1. The initial bacterial counts were 6.09 log CFU g⁻¹ for total viable count (TVC), 5.29 log CFU g⁻¹ for lactic bacteria (LAB), and 3.88 log CFU g⁻¹ for Staphylococcus spp, respectively. Relatively low bacterial counts in the Salami stuffing indicate a good hygienic quality of the raw materials. TVC, LAB and Staphylococcus spp counts had significantly increased during the ripening period (P<0.05). This increase in bacterial count is typical of most naturally dry fermented European sausages (Kozaciński et al., 2008). At the end of the Horse Salami production process, the mean values were 9.10, 7.79 and 5.10 log CFU g⁻¹, respectively.

As reported by many studies, microorganisms most represented during the ripening of cured sausages and meat products are LAB (Lizaso et al., 1999; Samelis and Georgiadou, 2000), whose counts tend to remain stable throughout the ripening period. Within the frame of this study, high LAB counts had been found during the first 28 ripening days, which can be related to a substantial pH drop witnessed during that period (Table 2). LAB inhibit the growth of pathogenic and spoilage bacteria by virtue of formation of lactic acid, acetic acid and possibly bacteriocins (Lucke, 2000).

**Isolation and identification of microbial population**

Native sausage products are of a higher quality than those obtained by virtue of controlled fermentation with the addition of industrial starters (Lebert et al., 2007). Many authors support the view that indigenous microflora or microorganisms present in traditional sausages originate from raw materials or the manufacturing environment (Maurello et al., 2004; Rantsiou et al., 2005). This microbiota is commonly referred to as “the house flora” (Garcia-Varoña et al., 2000).

Therefore, in this study, the isolation and identification of autochthonous microbial population inhabiting the Horse Salami was performed. The results of a microbiological analysis (Table 5) showed the dominant microflora to be the lactic acid bacteria strain termed Lactobacillus plantarum, Lactococcus lactis ssp. lactis, and Enterococcus faecium while the most represented coagulase-negative staphylococci strain was S. xylosus. The yeast Candida famata/Debaryomyces hansenii was found as well, which is in agreement with the results of Nielsen et al. (2008), who stated that halophilic yeasts most frequently isolated from fermented meat prod-
products are Debaromyces hansenii, Candida famata, Candida zeylanoides, Trichosporon sp., Cryptococcus sp. and Rhodotorula sp. Yeasts also play an important role in the maturation of sausages, since their lipolytic and proteolytic activity contributes to the development of sensory characteristics of fermented sausages (Kovačević, 2001; Alagić et al., 2008).

In the Horse Salami samples, bacteria of the Salmonella genus, Enterobacteriaceae, sulphite-reducing Clostridia, *L. monocytogenes* or *S. aureus* were not found; however, API biochemical tests uncovered the presence the *Listeria grayi* bacterium which is non-pathogenic (Table 5). Issues sometimes emerging with this type of fermented meat product are short shelf-life and poor hygienic surroundings, but the sausages produced in this investigation were proven to be microbiologically safe. It should be pointed out that biochemical (API) tests gave very good results (identification of one species with ID > 98.2-99.9 %).

The isolated lactic acid bacteria *L. lactis* ssp. *lactis, L. plantarum and E. faecium* could be used as starter cultures for meat products. *L. plantarum* as an autochthonous meat microflora is widely spread in nature (Salama et al., 1995; Ayad et al., 2001). *L. lactis* ssp. *lactis* in fermented sausage has rarely been reported so far and therefore further studies must to include detail molecular identification of isolated strains because API identification is not 100% precisely. The interest in exploring the potential of new strains isolated from different natural ecosystems to the effect of aroma compounds production has recently increased (Ayad et al., 2001; Frece et al., 2009; Babić et al., 2011, Frece et al., 2014). Metabolic properties of the *L. plantarum, E. faecium and L. lactis* species have both direct and indirect influence on organoleptic, nutritional and hygienic quality of fermented products. More and more research is focused on the isolation and identification of autochthonous functional starter cultures with the aim of developing new functional meat products that will be recognised and labelled as autochthonous to the region in which they are produced (Babić et al., 2011, Frece et al., 2014, Frece et al., 2014 a,b). Therefore, *L. plantarum, E. faecium, L. lactis* and *S. xylosus* as potential functional autochthonous starter cultures will be thoroughly investigated in the future. Further studies will be carried out to detail phenotypic, genotypic and physiological characterization of isolated strains of staphylococci and LAB.

### Sensory characteristics

Complex interaction between physicochemical, biochemical and microbiological processes, playing a role in formation of chemical compounds, and the modification of molecules responsible for the texture and appearance of the final product also determine its sensory characteristics. Average scores given by the panellists at the end of the Horse Salami manufacturing process are shown in Fig. 2. As for the external attributes, the Horse Salami scored highly when it comes to hardness (4.10±0.71) and low when it comes to appearance (3.60±0.43). It was highly rated for its sliceability, but low-rated when it comes to its colour intensity, fat/lean ratio and easy peeling capacity. After slicing, the highest scores were obtained for the fat distribution (4.78±0.67), while the fat/lean ratio scored low (2.22±0.44).

![Fig. 2 - Mean values of sensory properties of the final Horse Salami.](image_url)
Regarding the attributes that describe perceptions during mastication, Horse Salami was highly rated for its flavour intensity (4.10±0.44), juiciness (4.24±0.21) and acid taste (3.79±0.17), and low-rated for its saltiness and smokiness (2.41±0.31 and 2.20±0.19).

During the fermentation of dry sausages, LAB produce lactic acid (MATEO et al., 1996) responsible for the sour taste (LOTONG et al., 2000) and odour of the product, while mould odour is to be associated with 1-octen-3-ol, which spreads a typical mushroom odour (MEYNIER et al., 1998). In the present study, all three attributes scored highly (lactic acid taste 4.24±0.18; lactic acid odour 4.31±0.22; mould odour 3.82±0.15).

As for the smell descriptors, lactic acid (4.3±0.22) and mould odour (3.8±0.15) were dominant, while the spice odour scored low (3.00±0.28).

**Correlation between the parameters**

Instrumental colour parameters of the Horse Salami, established during its processing, were significantly inversely correlated (P<0.05) to the protein, fat, ash, collagen and salt content. Moisture content and $a_j$ values exhibited a significant direct correlation (P<0.05) to the instrumental colour parameters (Table 6). Relationships between the moisture, protein, fat, ash, collagen and salt content and $a_j$ on one hand, and hardness, gumminess and chewiness on the other, were also significant (P<0.05) (that between moisture and $a_j$ being an inverse one). Pearson’s correlation coefficients indicated that springiness and resilience are not significantly (P>0.05) correlated to the basic chemical composition, salt content and $a_j$ (Table 6).

**CONCLUSIONS**

This study investigated into the changes in physicochemical, colour, textural, microbiological and sensorial properties of the Horse Salami as an indigenous Croatian dry fermented sausage. During 90 days of manufacturing, major changes in physicochemical, colour and textural properties took place during the fermentation and ripening stage, pointing to proteolysis and lipolysis phenomena coming as a result of endogenous enzymatic activity, as well as to high lactic acid bacteria and staphylococci counts contributing to the specific organoleptic attributes of the final product. Sensorial profiling of the final Horse Salami showed a significant acid taste, lactic acid odour and flavour intensity, and low fat/lean ratio, saltiness and saltiness values. The final product was proven to be microbiologically safe, the dominant microbial population being L. lactis ssp. lactis, L. plantarum, E. faecium and S. xylosus.

**REFERENCES**


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Table 6 - Pearson’s correlation coefficients established between basic chemical composition, salt content, aw, texture and instrumental colour parameters.

<table>
<thead>
<tr>
<th></th>
<th>Moisture (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Collagen (%)</th>
<th>Ash (%)</th>
<th>Salt(NaCl) (%)</th>
<th>$a_j$</th>
<th>Springiness</th>
<th>Cohesiveness</th>
<th>Gumminess (kg)</th>
<th>Chewiness (kg)</th>
<th>Resilience</th>
<th>$L^*$</th>
<th>$a^*$</th>
<th>$b^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness (kg)</td>
<td>-0.95**</td>
<td>0.19</td>
<td>0.68**</td>
<td>-0.96**</td>
<td>-0.94**</td>
<td>-0.94**</td>
<td>0.48</td>
<td>0.95**</td>
<td>0.89**</td>
<td>-0.95**</td>
<td>-0.93**</td>
<td>-0.95**</td>
<td>0.95**</td>
<td>0.89**</td>
<td>0.94**</td>
</tr>
</tbody>
</table>

Values marked with ** are statistically significant (P<0.05).
material with a high level of mono and polyunsaturated fatty acids. Meat Sci. 80: 1182.


